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REVIEW ARTICLE

Discovering cardiac pericytes biology: from physiopathological mechanisms to potential therapeutic applications in ischemic heart disease

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Contribution for the special issue: "*Vascular biology: new mechanisms and pathways*"

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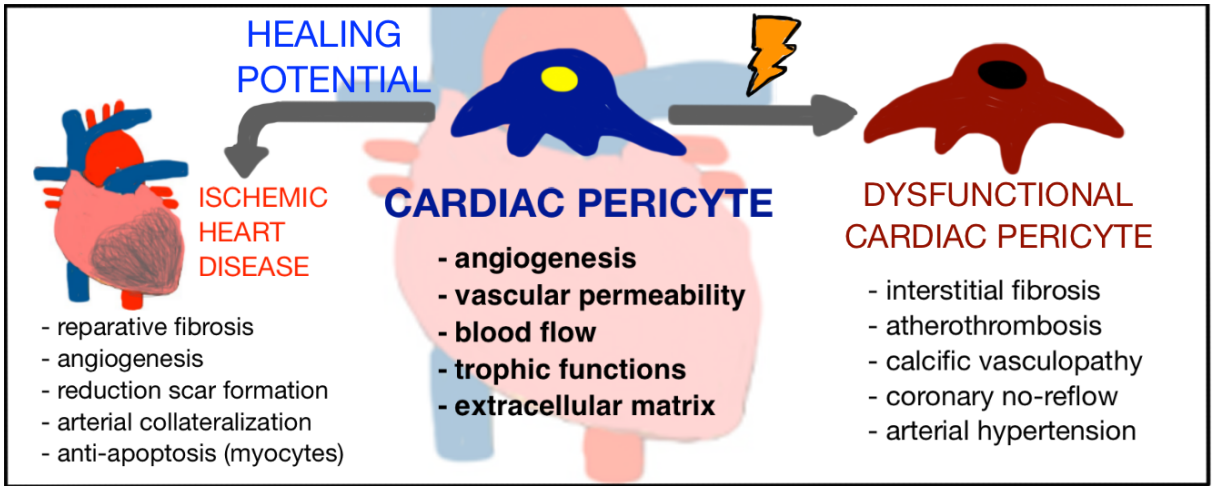
List of abbreviations

α-SMA	α-smooth muscle actin
ALK-1/5	activin receptor-like kinase 1/5
ALP	alkaline phosphatase
APs	adventitial pericyte-like progenitors
CPs	cardiac pericytes
CSCs	cardiac stem cells
ECM	extracellular matrix
ECs	endothelial cells
EMT	epithelial-to-mesenchymal transition
EPCs	endothelial progenitor cells
HGF	hepatocyte growth factor
LDL	low-density lipoprotein
MI	myocardial infarction/ischemia
MSCs	mesenchymal stem cells
NANOG	homeobox NANOG
NG2	neural/glial antigen 2
OCT4	octamer-binding transcription factor 4
PDGFR	platelet-derived growth factor receptor
PI3K	phosphoinositide 3'-kinase
SMCs	smooth muscle cells
SMPCs	smooth muscle progenitor cells
SOX2	(sex determining region Y)-box 2
SVPs	saphenous vein pericyte-like progenitors
TGF-β	trasforming growth factor-beta
VEGFA	vascular endothelial growth factor A
VEGFR2	vascular endothelial growth factor receptor 2
VSCs	vascular stem cells

Abstract

Microvascular pericytes and the more recently discovered adventitial pericyte-like progenitor cells are a subpopulation of vascular stem cells closely associated with small and large blood vessels respectively. These populations of perivascular cells are remarkably abundant in the heart. Pericytes control important physiological processes such as angiogenesis, blood flow and vascular permeability. In the heart, this pleiotropic activity makes pericytes extremely interesting for applications in regenerative medicine. On the other hand, dysfunction of pericytes could participate in the pathogenesis of cardiovascular disease, such as arterial hypertension, fibro-calcific cardiovascular remodeling, myocardial edema and post-ischemic coronary no-reflow. On a therapeutic standpoint, preclinical studies in small animal models of myocardial infarction have demonstrated the healing potential of pericytes transplantation, which has been ascribed to direct vascular incorporation and paracrine pro-angiogenic and anti-apoptotic activities. These promising findings open the door to the clinical use of pericytes for the treatment of cardiovascular diseases.

Graphical abstract



Keywords

Cardiac pericyte, Heart, Ischemic heart disease, Perivascular cell, Vascular stem cell, Adventitial progenitors, Myocardial infarction

1. Introduction

The heart is a complex organ and multiple classes of stem cells with different potential and lineage commitment are necessary to ensure the physiologic turnover of cells over time and contribute to the repair of the organ in case of injury. Endothelial cells (ECs) are the most frequent cell type into the heart, followed by cardiomyocytes [1]. So far, resident cardiac stem cells residing in myocardial niches have been extensively studied and used in animal models of cardiac diseases thanks to their supposed capacity to generate all the mature cell types forming the heart, from cardiomyocytes to vascular mural and endothelial cells. However, at the aim of achieving the revascularization of ischemic areas of myocardium, specialized cells able to promote both the phenomena of vasculogenesis (the formation of new vessels starting from a primitive cell) and angiogenesis (sprouting of new capillaries off of existing vessels through recruitment of new vascular cells) might represent the ideal candidates. Recently, the discovery of multipotent stem cells in the vasculogenic niche of blood vessels has opened new perspectives for cardiovascular regenerative therapies.

The vascular wall of small and large blood vessels is composed of different cell types with specialized functions, organized in concentric layers **(Fig 1)**. In all vessels, ECs line the luminal side in contact with the bloodstream, forming the so called *tunica intima*; among ECs functions, there are the regulation of vascular permeability and hemostasis, the control of the vascular tone and the recruitment and homing of leukocytes. Smooth muscle cells (SMCs) populate the central layer in small and big arteries, the *tunica media*; their major functions are the regulation of vessel contractility and the synthesis of new extracellular matrix. Finally, fibroblasts and other stromal cells give rise to the external layer of large vessels, the *tunica adventitia*, and their major function is the production of extracellular matrix (ECM) components. Importantly, the tunica adventitia has been recognized as a reservoir of vascular stem/progenitor cells, spatially organized in a specialized microenvironment, namely the vasculogenic niche.

Vascular stem cells (VSCs) are defined as multipotent cells residing within the blood vessels wall, or closely associated with the outmost layer of vessels, able to differentiate into all the cell types forming a mature functional vessel [2]. They are distributed along the entire vascular system, and their main functions are the maintenance of vessels integrity and the regulation of postnatal vasculogenesis. Based on the antigenic properties and differentiation capacity, VSCs are divided in four major populations: mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), smooth muscle progenitors cells (SMPCs) and perivascular cells, that are microvascular pericytes and adventitial pericyte-like progenitors **(Fig 1)** [3, 4]. MSCs, EPCs and SMPCs reside in the inner part of the tunica adventitia called *vasculogenic zone*, and in addition EPCs have been observed in the subendothelial layer at the interphase between the tunica intima and media of large vessels [5-8]. Instead perivascular cells can be found both within the vasculogenic zone but also around blood microvessels (see chapter 2). Noteworthy, despite the general definition given to VSCs, the differentiation potential varies among the cells, and not all VSCs are indeed able to generate all the cell types forming a blood vessel.

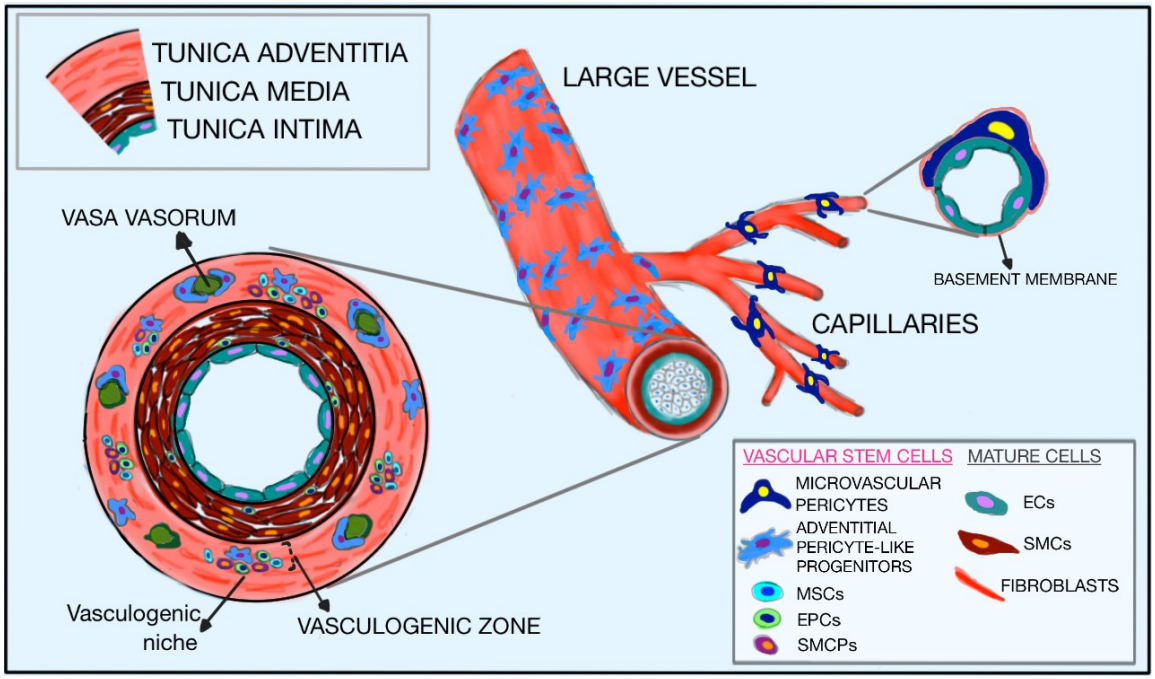


Figure 1: Vascular stem cells distribution in the vascular system. Cartoon showing the structure of capillaries and large vessels, and the localization of the different populations of vascular stem cells. *Abbreviations:* MSCs, mesenchymal stem cells; EPCs, endothelial progenitor cells; SMCPs, smooth muscle cell progenitors; ECs, endothelial cells; SMCs, smooth muscle cells.

This review will firstly introduce perivascular cells, also known as pericytes, reporting on their phenotype, classification and function; also, it will implement the classical concept of pericyte reporting on the new populations of adventitial pericyte-like cells recently described in large vessels. Secondly, it will focus on the heart and on cardiac pericytes (CPs), a population of cells with pleiotropic functions and whose role is gaining increasing importance in many biological processes. In particular, we will report on the dual and contrasting function of CPs, showing how they ensure the homeostasis of the organ in physiological conditions, while a perturbation of this state is reflected in cell dysfunction and the following contribution to cardiac pathologies, above all ischemic disease. In addition, we will show the promising therapeutic potential of pericytes derived from different sources – not only the heart – in *in vivo* preclinical studies for the treatment of ischemic heart disease.

2. Perivascular cells

Perivascular cells, commonly known with the name of pericytes, are a wide population phenotypically, biochemically and functionally heterogeneous. Their name derives from the peculiar localization surrounding and encircling endothelial cells of small blood vessels (*peri*, around; *cyto*, cell or vessel), where pericytes have been first identified.

While it was originally believed that the only role of pericytes was to support ECs contributing at the stabilization of existing blood vessels and promoting angiogenesis, recently pericytes have been reconsidered as important players in a series of biological processes, such as pathogenesis, regeneration and repair.

The concept of pericyte still represents an object of debate for Scientists. Originally, the ***microvascular pericyte*** has been defined as a cell encircling endothelial cells in capillaries and microvessels of most organs - excluded lymphatic vessels. However, in recent years, the discovery of cells with pericyte-like properties in the large vessels wall has challenged the unicity of the primitive concept, opening the way to a new population that here we name as ***adventitial pericyte-like progenitors (APs).*** These cells have been localized within the tunica adventitia of large vessels, sometimes associated with the *vasa vasorum* (nutritive microvessels in big veins and arteries) [9-12] **(Fig 1)**. Experimental evidences collected so far indicate that classical microvascular pericytes and APs might be considered stem cells because, similarly to MSCs, they are highly proliferative and clonogenic, express stemness markers and, importantly, they are endowed with capability of multilineage differentiation *in vitro* [13]. Proof of their multipotency is the myogenic [14, 15], neurogenic [16] and vasculogenic [9] potential, reflecting the peculiarity of the tissue of origin. In addition, because of their wide differentiation potential, pericytes have been considered the ancestors of MSCs [13].

To date, the relation between microvascular pericytes and APs has not been clarified yet. Although sharing similar phenotype and functional properties, pericytes and APs are characterized by different biological properties (see below). There is still a huge non-uniformity of opinion in describing and characterizing pericytes in different organs. In addition, the distinction between pericytes and MSCs or SMCs remains hard due to the high antigenic similarity. In the future, a general consensus similarly to what has been done for MSCs could help overcoming the current dilemma on how to establish the different identity of various cell populations sharing pericyte characteristics.

Multiple populations of pericytes and APs have been identified and characterized by several Authors in different human vessels and tissues, among which there are the saphenous vein [11], the skeletal muscle [14], the heart [9, 15], the adipose tissue [10], the retina [17], the bone marrow (*reticular cells*) [18] and the brain [16]. Also, specialized pericytes have been named as hepatic stellate cells or Ito cells in the liver [19] and mesangial cells in the kidney glomerulus [20].

2.1 The microvascular pericyte

Microvascular pericytes are contractile cells that support blood vessels by direct physical contacts with ECs and by release of paracrine molecular factors; they are placed under the basement membrane in close contact with ECs. Pericytes are characterized by multiple elongated cellular processes that wrap vessels; they communicate with neighboring ECs or other pericytes through peg-socket, gap and tight junctions [21-23]. Morphologically, *in situ* they are described like stellate cells with multiple cell processes encompassing the blood vessels. In the brain, the absence of pericytes leads to EC hyperplasia and abnormal structure, increase in vascular permeability and leakage, finally leading to hemorrhagic complications [24, 25], proof of a crucial contribution for pericytes in vascular homeostasis.

Microvascular pericytes have been extensively characterized thanks to the wide abundance around capillaries perfusing all the districts of the body. Their density around blood microvessels is highly variable depending on the organ and vascular bed; according to few single reports in the literature, the vasculature of the central nervous system and the retina is

characterized by the highest ratio pericytes to ECs (1:1), while in other tissues this density is definitely decreased (1:100 in the striated skeletal muscle) [26, 27].
Several important functions are known for microvascular pericytes, with specific functions developed based on the need of the organ in which they reside. On the one side, thanks to receptors for a large number of vasoactive signaling molecules, they control vascular caliber and tone and regulate vessel integrity and permeability; moreover, they support angiogenesis and EC differentiation and proliferation, produce extracellular matrix components and participate to the immune modulation [21, 22, 28]. On the other side, however, microvascular pericytes might also play a negative role promoting the occurrence of diseases; for example differentiation into myofibroblasts can implement a pro-fibrotic mechanism in organs like the muscles, the liver and the kidney [29, 30], while in the brain the release of pro-inflammatory factors by pericytes can activate microglial cells, finally resulting in neuro-inflammation [31].
Most of microvascular pericytes, independently from the different sources, share *in vivo* and in *ex vivo* cultures a common antigenic profile; this is given by several markers between CD44, CD73, CD90, CD105, platelet-derived growth factor receptor-β (PDGFR-β), neural/glial antigen 2 (NG2), desmin, vimentin, regulator of G-protein signaling 5, alkaline phosphatase (ALP) and stromal precursor antigen-1 [13, 21, 22, 32]. The expression of transcription factors proper of a multipotency or stemness state such as octamer-binding transcription factor 4 (OCT4), homeobox NANOG (NANOG) and (sex determining region Y)-box 2 (SOX2) has been occasionally documented [9, 11]. However, some discrepancies have been reported when describing pericytes from different Investigators, even within the same organ. In particular, the expression of the markers CD34 and CD146 distinguishes two different big populations of microvascular pericytes. One of them is characterized by a phenotype CD146/NG2^{pos}, CD34/CD31/CD45^{neg} [15], while the other one is described as CD34/NG2^{pos}, CD146/CD31/CD45^{neg} [9, 11]. This evidence underlines once again the huge heterogeneity of microvascular pericytes. Finally, microvascular pericytes do not express typical endothelial markers such as CD31 and von Willebrand Factor, and hematopoietic markers such as CD45.

2.2 The adventitial *pericyte-like* progenitor

The interest in APs has risen only recently, for this reason these cells have been less characterized then the microvascular counterpart. APs have been identified in the adventitia of the saphenous vein, myocardial vessels, internal thoracic artery, thoracic aorta and arteries/veins from other tissues, and described as CD34^{pos} but CD146/CD31/CD45^{neg} cells [9-11]. Markers like CD105, CD44, CD90, NG2 and PDGFR-β complete the phenotype of these pericyte-like cells.
Transplantation in small animals has confirmed that APs derived from the human saphenous vein are endowed with the characteristics of pericytes, given they re-assume a perivascular position and establish direct contacts with ECs [11, 33]. Although the specific role for APs has not been totally clarified yet, Scientists have hypothesized these cells would represent the real vascular stem cells, behaving like primitive cells whose main role is to act as progenitors for the generation of other more specialized cell types, including MSCs [8, 10, 13]. Therefore, APs would represent a functionally different and more primitive population than microvascular pericytes. Data in support of the “stemness hypothesis” is the evidence that

$CD34^{pos}$, $CD31/CD146^{neg}$ APs are multipotent at the clonal level, reason why they have been recognized as genuine ancestors of MSCs [10, 11]. In addition, they might be considered the precursors of microvascular pericytes [13, 34], although more work has still to be done in order to clarify the embryological origin and developmental hierarchy of all the mentioned cells.
Our group has demonstrated and then confirmed the great regenerative properties of human saphenous vein-derived APs in animal models of limb ischemia [11, 35, 36] and acute myocardial infarction (MI) [33, 37].
Finally, cells with similar characteristics have been identified also in the intimal sub-endothelium of the aorta and the saphenous vein [38], where they have been associated with the pathogenesis of atherosclerosis (see chapter 7).

2.3 How to identify pericytes?

Despite the increasing number of Investigators being focused on perivascular cells, no single marker able to unequivocally recognize these cells has been found so far, making the identification of pericytes still a challenge. This difficulty comes from the evidence that perivascular cells share several markers with other vascular resident cells, which are mainly MSCs but also SMCs and ECs [13, 39]. The colocalization inside vascular and perivascular niches and the similar morphology shown in *in vitro* cultures represent the reasons why for long time pericytes have been confused with MSCs; while the perivascular localization and the expression of smooth muscle alpha actin (α–SMA) easily confound pericytes with SMCs. In addition, the *in vitro* culture and expansion of cells may induce changes in the expression of some markers, making the characterization of any cell population even harder. For example, human $CD34^{pos}$, $CD31/CD146^{neg}$ pericytes have shown to down-regulate the expression of CD34 during the expansion [9, 11].
To date, the best identification of perivascular cells comes from a combination of criteria including the morphology, the anatomical localization *in situ*, the antigenic profile meant as the coexpression of two or more markers, the functional properties and the gene/protein expression pattern [22, 40].
The best confirmation of the nature of expanded cultures of pericytes comes from functional assays *in vitro*, which allow to distinguish pericytes from cells phenotypically similar such as MSCs [41]. A common functional test is the angiogenic cooperation with ECs, assessed with a matrigel assay in which pericytes promote and stabilize the formation of tubes made of ECs [11, 40]. Moreover, the assessment of cell-to-cell communication between pericytes and other vascular cells through the establishment of gap junction – commonly Connexin-43 – has been proposed as a test able to identify pericytes [42]. Also, the assessment of ALP enzymatic activity and the expression of pro-coagulatory components such as the tissue factor and the prothrombinase have been indicated as markers of the status of pericyte [42].

3. The cardiac pericyte

For long time several lines of investigations have been centred on the physiopathological role of pericytes in organs such as the retina, the kidney and the brain, while only recently an increasing interest has being shifted also to the heart. In fact, the recognition of the important

role of pericytes in cardiac homeostasis and physiopathology has induced more Investigators to focus on this cell type. This interest is further justified if we consider that pericytes are cells particularly abundant into a big demanding organ extensively vascularised like the heart. CPs play a central role in the organ homeostasis because they act as intermediate cells connecting the blood system and the vascular endothelium on the one side and the interstitial cells and cardiomyocytes on the other side.

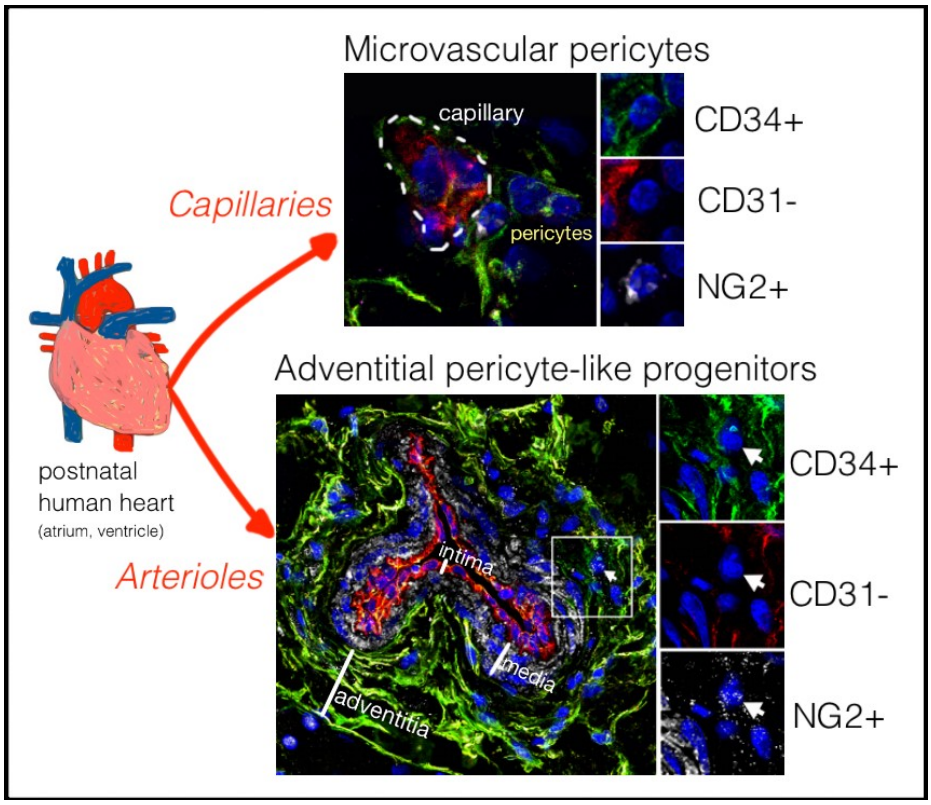


Figure 2: Perivascular cells in the postnatal human heart. Cartoon showing CD34+/CD31-/NG2+ microvascular pericytes and advential pericyte-like progenitors in the human neonatal heart. Images have been obtained from immunofluorescence staining of sections of myocardial tissue followed by confocal microscopy. Endothelial cells positive for the marker CD31 and/or CD34 line the lumen of both the capillary and the arteriole. The microvascular pericytes surround the capillary, while the adventitial pericyte-like cell is localized within the tunica adventitia of the arteriole. Vascular SMCs within the tunica media are recognized by the positivity to NG2. Images have been reproduced with permission of JAHA and taken from the original article in [9].

So far, only few populations of CPs deriving from the foetal and postnatal human heart have been expanded *in vitro* and extensively characterized. In 2011, Nees and colleagues have purified microvascular pericytes from the ventricular myocardium of adult explanted hearts [42]; in their work, Authors used a Percoll density-gradient centrifugation in order to select the population of interest after the enzymatic digestion of the tissue. Cultured pericytes were recognized by the expression of NG2, PDGFR-β, Connexin-43, α-SMA and Calponin; moreover they showed high expression of tissue factor and ALP activity. The morphology of the cells was the typical one of perivascular cells [42]. Later, at the beginning of 2015, the group of B. Péault identified a population of heart pericytes in myocardial samples from both

foetuses at 17-23 weeks of development and post-mortem adults [15]. Phenotypically, the expanded cells are NG2/PDGFR-β/CD146^{pos} but CD34/CD45^{neg}; they are able to differentiate into cells of the mesodermal lineage, including a limited capacity to generate cardiomyocyte-like cells both *in vitro* and *in vivo* in a mouse model of MI [15]. In the same period, our group has identified a population of microvascular pericytes and APs CD34/NG2/PDGFR-β^{pos} but CD31/CD45/CD146^{neg} in myocardial leftovers from neonates undergoing corrective surgery for congenital heart defects **(Fig. 2)** [9]. After expansion *in vitro*, cells down-regulate the expression of CD34, as we already reported previously in pericytes isolated from the saphenous vein [11]. Our CPs express also stemness markers (NANOG, OCT4 and SOX2), are clonogenic and show a marked plasticity toward the vascular SMC lineage, while being unable to originate electrically competent cardiomyocytes. Importantly, we showed that CPs are able to support the angiogenic activity of ECs in an *in vitro* angiogenic assay and are characterized by a secretome enriched with pro-angiogenic factors [9].

3.1 Epicardial pericytes are coronary SMCs progenitors

Recently Volz and colleagues used methods of clonal analysis and lineage tracing to elegantly demonstrate that epicardial pericytes in the mouse heart are progenitors of coronary artery SMCs [43]. Epicardial pericytes migrate to the myocardium during the coronary artery development and populate the coronary microvasculature; here, they participate to arterial remodelling. Specifically, in correspondence of the sites of vascular formation and remodelling close to the aorta, the arterial blood-flow induces Jagged-1 expression in the coronary cells. The crosstalk between the pericyte and the EC of the developing coronary vessel involves Notch3/Jagged-1 signalling. In response to Jagged-1, CPs upregulate Notch3 and differentiate into SMCs. Absence of arterial maturation in Notch3-null mice showed that Notch3 signalling is required for the pericyte-to-SMC transition [43]. In the adult heart, epicardial pericytes that are ancestors of SMCs and that take part in arterial remodelling have been identified as PDGFR-β/Notch3/NG2^{pos} but PDGFR-α^{neg} cells [43].

3.2 Ontogeny of cardiac pericytes

The developmental origin of the pericyte is still a sort of mystery, since the lack of absolute markers specific for pericytes has limited the studies of genetic fate tracing. However, some important information on the ontogeny of cardiac pericytes have been collected from studies with transgenic animal models.

The origin of perivascular cells in the heart can be traced back to epicardial mesothelial cells. A lineage analysis work by Cai and colleagues demonstrated that, in mice embryonic hearts, epicardial cells expressing the T-box transcription factor Tbx18 undergo epithelial-to-mesenchymal transition (EMT, a process crucial during early stages of embryo development [44]) and then invade the heart, where they give rise to fibroblasts and vascular mural cells, including both pericytes and SMCs coexpressing PDGFRβ; interestingly, ECs are Tbx18^{neg}, suggesting a different evolution [45]. At the same time, a study performed in mice with epicardial deletion of PDGFR-β demonstrated the importance of the PDGFR-β-phosphoinositide 3'-kinase (PI3K) axis signaling for the epicardial cells migration [46]. However, this study showed that mural cells develop also from a non-epicardial origin, although still dependent on PDGFR-β signaling [46]. In addition, PDGFR-β is required for

the epicardial cell EMT [47]. Finally, the Wilm tumor suppressor 1 (Wt1)/Wnt/β-catenin signaling pathway is another important axis able to promote the epicardial EMT, ensuring the correct cell spindle orientation and stabilizing the adherens junctions [48, 49].

4. Cardiac pericytes signaling and crosstalk with neighboring cells help to preserve heart physiology

Pericytes within the heart participate in many processes regulating cardiac homeostasis. Phenomena such as angiogenesis, the regulation of blood flow, vascular maturation and permeability, delivery of trophic substances from the systemic circulation into the myocardial interstitium, and the regulation of the coagulatory process are only some of the multiple functions attributable to pericytes **(**illustrated in **Fig. 3)** Some aspects related with these mechanisms are summarized below.

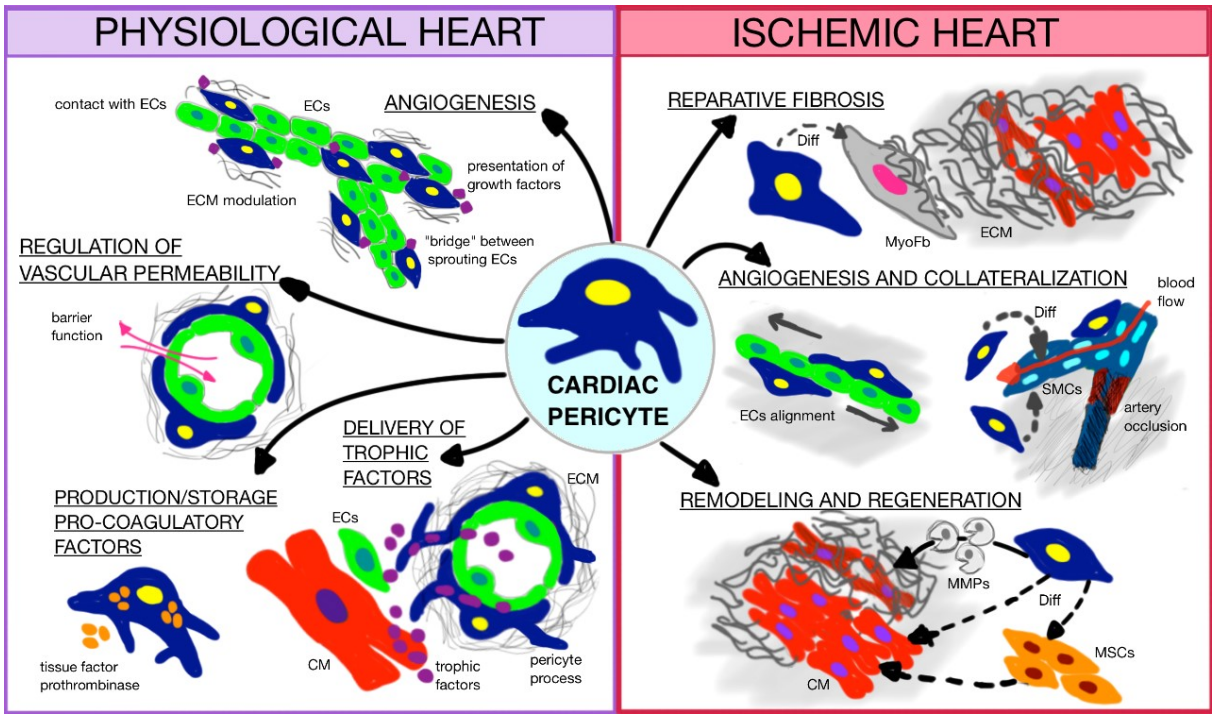


Figure 3: Cartoon summarizing the functions of cardiac pericytes in the physiological and pathologic ischemic heart. For details and explanation, see chapter 4 and 5. *Abbreviations:* CM, cardiomyocyte; Diff, differentiation; ECs, endothelial cells; ECM, extracellular matrix; MMPs, metalloproteinases; MSCs, mesenchymal stem cells; MyoFb, myofibroblast.

4.1 Angiogenesis, regulation of vessel maturation and permeability

Pericytes are crucial regulators and stabilizers of angiogenesis and vessel permeability, with the physical or paracrine interaction between ECs and pericytes playing a primary role in this process. When required, pericytes undergo the transition from a quiescent to the angiogenic state, which involves multiple changes in pericytes and the modifications in the contact with ECs: pericyte migration and proliferation, cell maturation, coverage of ECs, presentation of

growth factors and modulation of extracellular matrix [22, 39]. On the other side, EC viability, proliferation and angiogenic activity are regulated. In general, independently from the organ, the interaction between the pericyte and the EC is regulated by the crosstalk between some ligands/receptors, the main of which are Angiopoietin-1 (Ang1)/Tie2, transforming growth factor-β (TGF-β)/ALK-1/5, Vascular Endothelial Growth Factor A (VEGFA)/VEGFR2, PDGF-BB/PDGFR-β, N-Cadherin/N-Cadherin, Jagged1/Notch **(Fig. 4).** Ligands can be either soluble (released by cells) or exposed onto the cell membrane. The **Table 1** summarizes the processes regulated by each molecular interaction.

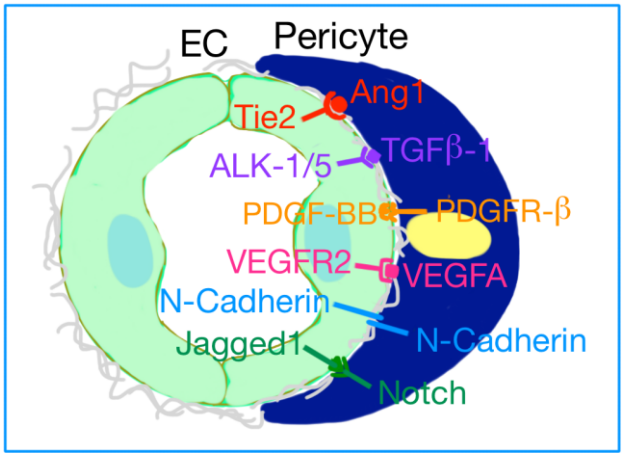


Figure 4: Cartoon summarizing the most characterized molecular interactions occurring between ECs and pericytes. *Ligands can be soluble (paracrine factors) or presented on the cell membrane.*

Ligand/Receptor	Process regulated	Ref
Ang1/Tie2	Vessel stabilization (anti-leak)	[50]
TGFβ/ALK-1/5	EC proliferation and migration (ALK-1) Vessel maturation (ALK-5)	[51]
PDGF-BB/PDGFR-β	Pericyte recruitment and proliferation Vessel stabilization	[52] [50]
VEGFA/VEGFR2	ECs survival Vessel stabilization Angiogenesis sprouting	[53] [53] [54]
N-Cadherin/N-Cadherin	Pericyte recruitment Vessel maturation	[55]
Jagged1/Notch	Vessel maturation and stabilization Angiogenesis sprouting	[56]

Table 1. Summary of the processes favoring angiogenesis regulated by the main physical or paracrine interactions between ECs and pericytes.

Also, it has been documented that pericytes can bridge the temporary gaps created between two sprouting EC segments, in this way supporting the angiogenic process [57]. However, the concept of pericytes being vascular stabilizers has been questioned. Bodnar and colleagues showed that human skeletal muscle pericytes do not support the angiogenic process, rather

causing a regression of the endothelial tubes formed in an *in vitro* matrigel assay; the mechanism described involves the activation of μ-calpain – protease responsible for the cleavage of integrins - induced by the interaction between the CXC chemokine receptor 3 (CXCR3) expressed on ECs membranes and its ligand produced by pericytes [58].
The recruitment of pericytes to the developing vasculature is an important step in blood vessel maturation [59]. The receptor tyrosine kinase TrkB expressed on the surface of pericytes plays an important role in vessel maturation; the ligand for TrkB is the brain-derived neurotrophic factor (BDNF), produced by ECs. The signalling downstream BDNF/TrkB is critical for the the correct development of the endothelial-pericyte barrier, as demonstrated by studies in TrkB deficient mice, which show a reduced pericytes coverage of the cardiac microvasculature, an abnormal endothelium and increased vascular permeability [59].
In addition to the common microvascular pericytes, CPs have been identified also around the pre-capillary arteriolar and post-capillary venular walls, instead of the expected vascular SMCs, sites in which they play a crucial role in the regulation of the blood flow [60]. Different properties are attributed to these cells. While around arterioles pericytes are numerous and embedded in a thick ECM whose function is the maintenance of the vascular wall tightness, the venular pericytes are less abundant, fragile and characterized by a weakly developed ECM [60]. Cross-talk between ECs and pericytes in both coronary pre-capillary arterioles and post-capillary venules is crucial for the maintenance of vascular wall tightness and regulation of permeability [61].

4.2 Trophic functions

In addition to the trophic support for ECs common to all pericytes, an important function of CPs might be the regulation of the barrier endothelial cells-pericytes and pericytes-ECM allowing the flux of nutritive substances - such as the fatty acids required for the demanding cardiomyocyte metabolism - from the circulating blood into the interstitium, and thus to cardiomyocytes. In order to overcome the hydrophobic ECM, pericytes processes pass through the ECM and build connections with other interstitial cells through gap junctions.

4.3 Production of pro-coagulatory substances

Pericytes are the only cells in the vascular wall producing tissue factor [62]; moreover, they produce prothrombinase [42]. Arteriolar and coronary artery pericytes control coagulatory processes by incorporating and storing tissue factor and prothrombinase at high levels in their plasmalemma and ECM; instead, venular pericytes produce less amounts of the substances above in physiological conditions but significantly upregulate their expression when an inflammation occurs [42].

5. Healing potential of local pericytes in the ischemic heart

Ischemic heart disease is characterized by a reduced blood supply to the cardiac muscle. One of its severe manifestations, the acute MI, is characterized by the sudden occlusion of a coronary artery; the consequence is an acute loss of blood flow to a region of myocardium, resulting in a massive necrosis of myocytes. The infiltration of circulating inflammatory cells and the proliferation of myofibroblasts promote the reabsorption of the necrotic tissue, which

is gradually substituted by “reparative fibrosis”, important to preserve the structure of the heart although at the expense of an increase in stiffness. The final effect is a change in the shape and architecture of the left ventricle that usually becomes more dilated, process named adverse ventricular remodelling. If this process becomes uncontrolled and persistent over time, the function of the heart as a pump will be impaired, finally bringing to a global failure of the whole organ [63].

In the contest of a MI, what we need are: 1) the substitution of necrotic areas with fibrotic tissue (process called “repair”), in order to preserve the structural integrity of the organ and 2) the revascularization of the damaged areas of myocardium, since without perfusion of blood bringing oxygen and trophic factors no vital tissue can grow. In later stages, 3) ideally the fibrotic tissue should be degraded and lost cells be replaced by new functional cells (process named “regeneration”). Given their pleiotropic properties, pericytes might be able to address all these purposes in the ischemic heart, from the organ repair to its later regeneration **(Fig. 3)**.

How do pericytes work? Starting from the first point, pericytes can directly differentiate into myofibroblasts producing reparative fibrosis that can contribute to the preservation of the ventricular structure in early phases post-MI. This process implements the repair through granulation that occurs thanks to tissue macrophages.

For the revascularization, CPs can recruit ECs to the site of injury by paracrine mechanisms; the physical cooperation between CPs and ECs is crucial to instruct ECs to organize in vascular structures, favouring the angiogenic process. In addition, pericytes can attract and recruit other pericytes, amplifying the reparative process. The documented ability of CPs to differentiate into vascular SMCs [9, 43] may also promote arterial collateralization, term indicating the formation of new alternative vascular branches that overcome the point of an artery obstruction and perfuse the downstream myocardial tissue. This event is important in the peri-infarct myocardium, since the arteriogenesis boosts the reperfusion of ischemic zones.

With regard to the last point listed above, scar remodelling and tissue regeneration, CPs might release tissue metalloproteinases able to degrade the fibrous matrix, as it has already been demonstrated in other organs. Ideally, for the optimal healing of the ischemic heart it would be crucial that the degradation of the fibrotic scar was followed side-by-side by the generation of new cardiomyocytes and other stromal cells. Although this process is not sufficient in case of extensive injury, the cardiomyogenic ability of CPs has recently been documented [15]. Given the abundance of microvascular pericytes in the heart compared with other cardiac stem cells (CSCs), the cardiomyogenic and vasculogenic contribution of resident CPs might be the key for the resolution of limited damages post-ischemia, especially in non-severe forms.

Last, the supposed role of precursors of MSCs attributed to vascular pericytes [64] underlines once again the importance of these cells in ischemic situations, given the need of various populations of progenitor cells with different lineage commitment for the repair of the functionally complex heart tissue.

6. Therapeutic effect of pericytes transplantation in preclinical models of myocardial infarction

Pericytes are gaining an increasing interest for application in cardiovascular regenerative medicine due to their pleiotropic properties and strong angiogenic capability; the reasons for

using these cells are several. Importantly, first of all pericytes are safe cells, since they overcome the teratogenic risk associated with pluripotent stem cells; however, despite preclinical studies have not raised concerns regarding side effects of pericytes, a systematic tumorogenesis investigation needs to be done before starting clinical trials according to international guidelines. Then, their rich secretome is composed of cytokines and growth factors able to stimulate the process of tissue regeneration and angiogenesis by paracrine mechanisms, and of chemoattractant molecules able to recruit further cells to the site of injury. Moreover, the relative abundance of pericytes can be an advantage when choosing the best cell type to use for cell therapy. As an example, microvascular CPs are much more abundant than other classes of stem or progenitor cells in the heart; as a consequence, the process of isolation and expansion of pericytes from small cardiac biopsies might be easier than the derivation of classical CSCs. At this regard, we demonstrated the feasibility and high reproducibility of deriving CPs, then expanded to several millions, starting from tiny myocardial leftovers weighing only less than 100 mg, obtained from new-borns undergoing corrective surgery for congenital heart defects [9]. A further advantage of using pericytes for cell therapy applications might derive from the supposed immune-privileged nature of the cells; if confirmed true, this factor will support and favour the allogeneic transplantation when the urgency to receive the cell therapy does not allow enough time for the autologous approach.

The cell therapy approach is based on the isolation and expansion *in vitro* of pericytes to reach a number sufficiently high for autologous cell therapy into the same patient. At this purpose, on the one side pericytes deriving from extra-cardiac sources might be preferred for the easy accessibility to peripheral tissues, and have been already used in animal models of ischemic heart disease. On the other side, however, pericytes deriving from the heart might be endowed with higher plasticity and commitment versus all the cardiovascular lineages.

6.1 Preclinical studies with non-cardiac pericytes

A limited number of studies performed recently have tested the therapeutic activity of *non-cardiac pericytes* in preclinical models of MI, but this field is intended to grow quickly in the upcoming years. In all the studies reported below, the major benefits of pericytes are attributable to their paracrine activity rather than to a direct trans-differentiation into cardiovascular cells or the fusion with resident cardiac cells. The first application can be traced back to 2011, when our group demonstrated the great regenerative ability of human saphenous vein derived pericyte-like progenitors (SVPs) in both an immunocompetent and immunodeficient mouse model of MI [33]. When delivered in the peri-infarct area, SVPs were able to improve the indexes of cardiac function and contractility, to reduce the size of the fibrotic scar and to stimulate angiogenesis compared with control mice not receiving cell therapy. The mechanistic pathway is based on the microRNA-132, constitutively released by SVPs but further upregulated under hypoxia; importantly, microRNA-132 reduces the differentiation of fibroblasts into myofibroblasts, through the inhibition of Ras-GTPase activating protein and methyl-CpG-binding protein 2, thus exerting a protective action from fibrosis [33]. The peculiar resilience of SVPs to oxidative stress [36] may promote angiogenesis in the infarcted ventricle. Two years later, Chen and colleagues tested the regenerative ability of human microvascular skeletal muscle pericytes in an immunodeficient

mouse model of MI [65]. Intramyocardial transplantation of CD146^{pos}, CD34/CD45/CD56^{neg} pericytes improved left ventricular function and angiogenesis, while attenuating myocardial fibrosis and the infiltration of inflammatory cells into the site of injury, compared to controls [65]. In the same year, Yannarelli and colleagues evaluated the reparative ability of human umbilical cord perivascular cells in an immunodeficient mouse model of MI [66]. Similarly to the previous studies, pericytes improved indexes of cardiac contractility; however, no improvement was observable with regard to infarct size in comparison with control mice [66].

6.2 Preclinical studies with cardiac pericytes

The first preclinical study with CPs was published only recently, at the beginning of 2015, from the group of Bruno Péault [15]. Contrary to cells sourced from other tissues, a subpopulation of CPs (already described in chapter 3) was able to generate new cardiac Troponin I and T and Connexin-43^{pos} cardiomyocytes when injected either in infarcted ventricles of immunodeficient mice or in healthy animals, demonstrating their cardiomyogenic potential [15]. Once again, it might be possible that pericytes deriving from the heart are more committed toward a cardiogenic process. But this needs still to be demonstrated given pericytes heterogeneity. For example, given their vasculogenic properties, CPs might be the ideal candidate for vascular application within the heart as compared with pericites from other organs. Furthermore, our studies show that cardiac pericytes do not have spontaneous electrical activity and therefore it is unlikely that once transplanted into the heart they will trigger arrhythmias [9].

6.3 Combinatorial cell therapy

Another intriguing possibility to reach better results in terms of cardiac regeneration is the combinatorial cell therapy, consisting in the simultaneous delivery of multiple cell types to improve the processes of cardiomyogenesis and vasculogenesis in the injured heart. A study of this kind was recently performed by our team [37]. The combined intramyocardial delivery of human SVPs and cKit^{pos} CSCs in an immunodeficient mouse model of MI resulted in the enhancement of the cell therapeutic activity in comparison with the cell populations delivered in single therapy. In fact, in our model SVPs and CSCs additively work to reduce the infarct size and to stimulate vascular proliferation and arteriogenesis in the infarcted left ventricle, although cardiac function did not show a further improvement compared with the effects of the single cell populations [37].

6.4 Limitations and future studies

Although positive and encouraging results have been obtained with the above preclinical models of MI, the limitation of these studies is represented by the use of immunodeficient mice, in which the immune system is compromised and thus leaves space to questions and doubts on the real therapeutic activity of cells when translated into a clinical scenario. Considering this point, it is clear that further studies with pericytes in large animal models of MI, for which the physiological anatomy of the cardiovascular system is much closer to that of humans, are required before proceeding with any clinical trial. This will be the next challenge. In the meanwhile, moving toward a clinical translation of pericyte products, our

team has optimized a standard operating procedure for the production of clinical grade SVPs for application in patients with refractory angina [67].

7. Pericyte dysfunction and implication in cardiovascular diseases

Cells are receptive elements that actively respond to changes in the physiological environment in which they reside; chemical or mechanical stimuli can perturb cell behaviour and trigger particular intracellular pathways resulting in local or systemic phenomena. These responses can be positive but also injure the organism. Also for CPs, despite the marked healing properties described above, a perturbation of the cell physiological behaviour can activate quiescent mechanisms and transform cells in dangerous elements participating in the pathogenesis of cardiac diseases. Molecular programs regulating these dual fates of pericytes are yet unknown, but it is reasonable that different subtypes of pericytes might be involved. The multiple functions of CPs and the wide number of biological processes in which they are involved might even favour this negative transformation, through exposure of cells to different stressful stimuli.

When activated, dysfunctional pericytes may differentiate into chondrocytes, osteocytes, macrophages and myofibroblasts, contributing to the onset of phenomena such as atherosclerosis, fibrosis and vascular calcification **(Fig 5)**. As follows, we report on the main findings relative to these mechanisms.

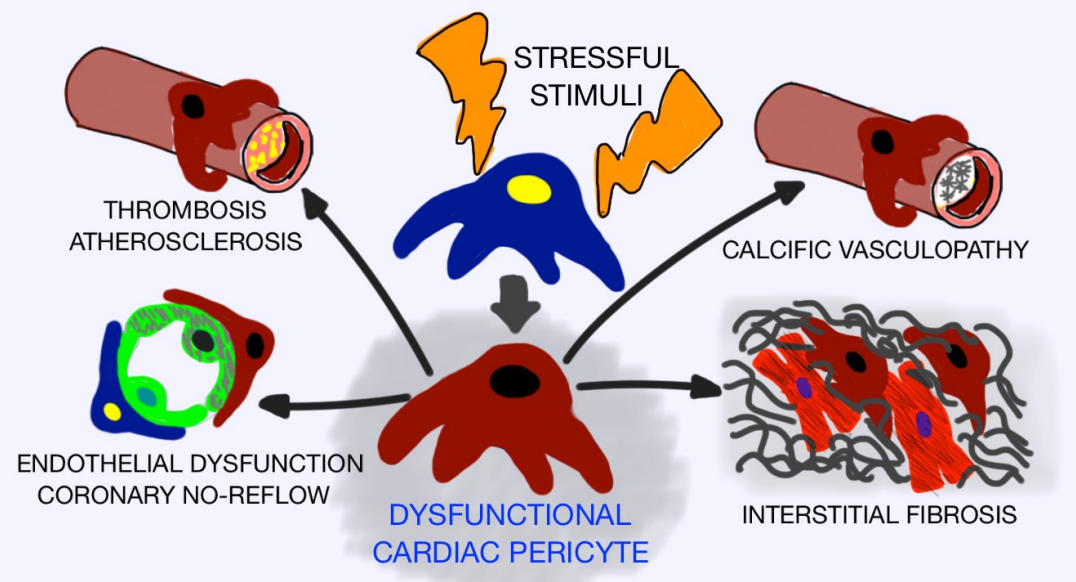


Figure 5: Cartoon summarizing the contribution of dysfunctional pericytes in the pathogenesis of cardiovascular diseases.

7.1 Thrombosis and atherosclerosis

The intra-operatory preparation of explanted saphenous veins before coronary artery bypass surgery requires the non-pulsatile flushing of the vein with various types of saline solutions, procedure that causes the de-endothelialization of up to 75% of the total endothelial surface; this situation triggers coagulatory processes involving the release of tissue factor and

assembling of prothrombinase in the subendothelium. This protocol has been indicated as responsible for the failure of the bypass graft [68]. Indeed, a contribution of macrovascular intimal pericytes in the pathogenesis of atherosclerosis, thrombosis and saphenous vein graft failure has been described. Juchem et al. identified a population of pericytes expressing high concentrations of tissue factor in the sub-endothelium of the aorta and the saphenous vein, coupled with ECs of the luminal intima [38]. Importantly, these pericytes were found within atherosclerotic plaques and coronary vein grafts stenosis, in which were responsible of the intima thickening and represented more than the 90% of proliferating cells. The exposure of endothelium-denudated venous grafts to circulating serum provoked a significant increase in the amount of tissue factor (up to 25-folds in 1 hour in *in vitro* tests), highlighting a potent pro-coagulatory and pro-thrombogenic ability for intimal pericytes [38]. In healthy vessels, however, the intact luminal endothelium exerts a physiological protective and preventive function, neutralizing the tissue factor through the system thrombomodulin-protein C and avoiding the occurrence of thrombosis [38].

A mechanism responsible for the recruitment of pericytes to the site of the developing atherosclerotic lesion could involve the PI3K/Akt axis activated by the binding of the hepatocyte growth factor (HGF) to its receptor c-Met on the surface of pericytes [69]. Although this mechanism has been described in femoral arteries, it is possible that the same pathway is involved in the heart, where HGF is produced by human aortic ECs and vascular SMCs [70]. T-cadherin, a unique member of the cadherin family of adhesion molecules highly expressed in the aorta, is another factor implicated in the atherosclerotic process. An analysis of this factor in normal human aorta and atherosclerotic lesions showed that T-cadherin is expressed by ECs, SMCs and pericytes in the aortic intima and media, and in the walls of *vasa vasorum* and pericytes in the adventitia. Noteworthy, its expression in SMCs and pericytes correlates with the severity of the lesion, with higher levels of T-Cadherin corresponding to more severe stages of atherosclerosis [71]. Last, in the human atherosclerotic coronary artery, a mutation in low-density lipoprotein (LDL) receptor-related protein 6 causes early atherosclerosis; this mutation co-localizes with the PDGFR-β in vascular SMCs and induces cell proliferation in response to PDGF stimulation [72]. Given the high expression of PDGFR-β in pericytes, it is likely that this pathway might be triggered by PDGF also in these cells.

7.2 Ischemia/reperfusion injury

Studies performed with a mouse model of cardiac ischemia-reperfusion injury have demonstrated that microvascular pericytes are responsible for the occurrence of endothelial dysfunction with a mechanism involving the neurotrophine receptor $p75^{NTR}$ [73]. This mechanism would work also in human subjects. During ischemia, cardiomyocytes rapidly increase the secretion of pro-Nerve Growth Factor, which binds to the $p75^{NTR}$ on the surface of PDGFR-β^{pos} pericytes and this results in a modification of pericytes architecture including a reduction of vascular coverage and a shortening of cell processes, finally disrupting the endothelial cell-pericyte interaction and provoking an increase of vascular permeability; the cardiomyopathy becomes lethal in adult mice [73].

Another phenomenon involving pericytes occurring in ischemic patients is the coronary no-reflow, described as the failure to achieve adequate reperfusion of the cardiac

microcirculation after the reperfusion that follows a coronary artery obstruction. It represents a common complication of the reperfusion procedure. Because of constriction mechanisms, cardiac pericytes have been identified as responsible for the failed coronary capillaries reperfusion [74].

7.3 Calcific vasculopathy

One possible mechanism responsible for the chondrogenic differentiation of pericytes, described in retinal pericytes, involves the Wnt/β-catenin signalling [75]. Pericytes express several Wnt receptors, among which there are the LDL receptor-related proteins 5 and 6 and some receptors of the Frizzled family. TGF-β3 is an activator of the Wnt/β-catenin pathway, which induces the expression of the chondrocyte marker SOX-9 and the accumulation of collagen and glycosaminoglycan into the pericyte matrix [75]. Importantly, in the heart SMCs, macrophages and foam cells within aortic atherosclerotic plaques produce high levels of TGF-β3 [76], thus this mechanism might promote the aortic calcification. Another factor associated with the osteogenic differentiation of pericytes is the Vascular Calcification Associated Factor (VCAF), detectable in arterial calcified lesions but not in noncalcified arteries, and highly expressed during the osteogenic differentiation of pericytes *in vitro*. Interestingly, VACF has been described as a protective factor in pericytes, since its knockdown resulted in accelerated mineralization *in vitro* and increased formation of calcific nodules [77]. VACF could be upregulated as a defence mechanism against vascular calcification. SMCs, ECs, macrophages and osteoblasts within femoral calcified arteries would be the cells responsible for VACF production [78]. Although this factor has been investigated in femoral arteries, we can suppose that a similar mechanism might regulate the evolution of calcific lesions into cardiac arteries.

7.4 Fibrosis

The role of pericytes in myocardial fibrosis and perivascular fibrosis has not been elucidated yet. Despite the cellular origin of myofibroblasts has been unclear for many years, we presently know that perivascular cells might differentiate into myofibroblasts [79]. What is known is that cardiac pericytes produce proteins of the ECM [42]. As said above (chapter 5), substitutive fibrosis can be beneficial to the organ after a MI occurs; however, if this process becomes uncontrolled, the persistent deposition of interstitial fibrosis and increase in ventricular wall stiffness will be detrimental for the global heart function.
In the mouse heart $Gli1^{pos}$ perivascular adventitial cells have been identified as precursors of myofibroblasts and indicated as responsible for heart fibrosis after an injury, finally leading to heart failure [80]. The genetic ablation of $Gli1^{pos}$ cells was able to rescue the organ function, suggesting that this subpopulation of perivascular cells might be the therapeutic target for the prevention of organ dysfunction [80]. In heart failure, galectin-3 released by inflammatory macrophages activates, in a paracrine fashion, other macrophages, fibroblasts and pericytes; the result of this stimulus is the production of pro-collagen I, which is finally converted into the mature and cross-linked forms contributing to myocardial fibrosis [81].
Another work showed that in a mouse model of Duchenne muscular dystrophy the accumulation of severe coronary perivascular fibrosis is caused by a population of adventitial Sca1/PDGFR-α^{pos}, CD31/$CD45^{neg}$ cells producing high levels of Collagen type I in response

to TGFβ1 signalling [82]. Wu and colleagues published similar results, demonstrating that adventitial perivascular Sca1^{pos} cells residing in the murine aorta contribute to the fibrotic remodelling in hypertension [83].
However, contrasting results have been obtained. Recently, Birbrail et al. demonstrated that Nestin/NG2 ^{pos} pericytes significantly accumulates in the mouse infarcted area post-MI; however, these cells do not produce type I Collagen and thus do not contribute to the formation of the fibrotic scar. Collagen was instead produced by NG2^{neg} cells [84]. In support of this evidence, our group reported that NG2^{pos} pericytes isolated from the human saphenous vein and injected intra-myocardially in the site of injury in a mouse model of MI are protective against fibrosis (see chapter 6) [33].

8. Conclusion

Despite the recognized biological potential of pericytes and APs residing in the heart, so far these cells have been under-investigated in applications for cardiovascular therapy and regeneration. But the current research is paving the way for future studies. Also, both the crucial involvement of pericytes in the control of cardiac physiology and the contribution of dysfunctional pericytes to pathological conditions of the heart are elements underlining the need to focus more on this population of cells, since new mechanistic insights might derive from the knowledge of the biology of CPs and, as a consequence, new therapeutic approaches might be developed for cardiac diseases.

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References

1. Pinto, A.R., et al., *Revisiting Cardiac Cellular Composition.* Circ Res, 2016. **118**(3): p. 400-9.
2. Lin, C.S. and T.F. Lue, *Defining vascular stem cells.* Stem Cells Dev, 2013. **22**(7): p. 1018-26.
3. Bobryshev, Y.V., A.N. Orekhov, and D.A. Chistiakov, *Vascular stem/progenitor cells: current status of the problem.* Cell Tissue Res, 2015. **362**(1): p. 1-7.
4. Psaltis, P.J. and R.D. Simari, *Vascular wall progenitor cells in health and disease.* Circ Res, 2015. **116**(8): p. 1392-412.
5. Ingram, D.A., et al., *Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells.* Blood, 2005. **105**(7): p. 2783-6.
6. Zengin, E., et al., *Vascular wall resident progenitor cells: a source for postnatal vasculogenesis.* Development, 2006. **133**(8): p. 1543-51.
7. Pasquinelli, G., et al., *Multidistrict human mesenchymal vascular cells: pluripotency and stemness characteristics.* Cytotherapy, 2010. **12**(3): p. 275-87.
8. da Silva Meirelles, L., A.I. Caplan, and N.B. Nardi, *In search of the in vivo identity of mesenchymal stem cells.* Stem Cells, 2008. **26**(9): p. 2287-99.
9. Avolio, E., et al., *Expansion and characterization of neonatal cardiac pericytes provides a novel cellular option for tissue engineering in congenital heart disease.* J Am Heart Assoc, 2015. **4**(6): p. e002043.
10. Corselli, M., et al., *The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells.* Stem Cells Dev, 2012. **21**(8): p. 1299-308.
11. Campagnolo, P., et al., *Human adult vena saphena contains perivascular progenitor cells endowed with clonogenic and proangiogenic potential.* Circulation, 2010. **121**(15): p. 1735-45.
12. Klein, D., et al., *Vascular wall-resident CD44+ multipotent stem cells give rise to pericytes and smooth muscle cells and contribute to new vessel maturation.* PLoS One, 2011. **6**(5): p. e20540.
13. Crisan, M., et al., *A perivascular origin for mesenchymal stem cells in multiple human organs.* Cell Stem Cell, 2008. **3**(3): p. 301-13.
14. Dellavalle, A., et al., *Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells.* Nat Commun, 2011. **2**: p. 499.
15. Chen, W.C., et al., *Human myocardial pericytes: multipotent mesodermal precursors exhibiting cardiac specificity.* Stem Cells, 2015. **33**(2): p. 557-73.
16. Nakagomi, T., et al., *Brain vascular pericytes following ischemia have multipotential stem cell activity to differentiate into neural and vascular lineage cells.* Stem Cells, 2015. **33**(6): p. 1962-74.
17. Zhong, Y., J.J. Wang, and S.X. Zhang, *Intermittent but not constant high glucose induces ER stress and inflammation in human retinal pericytes.* Adv Exp Med Biol, 2012. **723**: p. 285-92.
18. Madelung, A., et al., *A novel immunohistochemical sequential multi-labelling and erasing technique enables epitope characterization of bone marrow pericytes in primary myelofibrosis.* Histopathology, 2012. **60**(4): p. 554-60.
19. Hellerbrand, C., *Hepatic stellate cells--the pericytes in the liver.* Pflugers Arch, 2013. **465**(6): p. 775-8.
20. Kramann, R. and B.D. Humphreys, *Kidney pericytes: roles in regeneration and fibrosis.* Semin Nephrol, 2014. **34**(4): p. 374-83.

21. Dore-Duffy, P. and K. Cleary, *Morphology and properties of pericytes.* Methods Mol Biol, 2011. **686**: p. 49-68.
22. Armulik, A., G. Genove, and C. Betsholtz, *Pericytes: developmental, physiological, and pathological perspectives, problems, and promises.* Dev Cell, 2011. **21**(2): p. 193-215.
23. Diaz-Flores, L., et al., *Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche.* Histol Histopathol, 2009. **24**(7): p. 909-69.
24. Armulik, A., et al., *Pericytes regulate the blood-brain barrier.* Nature, 2010. **468**(7323): p. 557-61.
25. Hellstrom, M., et al., *Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis.* J Cell Biol, 2001. **153**(3): p. 543-53.
26. Mathiisen, T.M., et al., *The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction.* Glia, 2010. **58**(9): p. 1094-103.
27. Shepro, D. and N.M. Morel, *Pericyte physiology.* FASEB J, 1993. **7**(11): p. 1031-8.
28. Orekhov, A.N., Y.V. Bobryshev, and D.A. Chistiakov, *The complexity of cell composition of the intima of large arteries: focus on pericyte-like cells.* Cardiovasc Res, 2014. **103**(4): p. 438-51.
29. Greenhalgh, S.N., J.P. Iredale, and N.C. Henderson, *Origins of fibrosis: pericytes take centre stage.* F1000Prime Rep, 2013. **5**: p. 37.
30. Schrimpf, C. and J.S. Duffield, *Mechanisms of fibrosis: the role of the pericyte.* Curr Opin Nephrol Hypertens, 2011. **20**(3): p. 297-305.
31. Matsumoto, J., et al., *Tumor necrosis factor-alpha-stimulated brain pericytes possess a unique cytokine and chemokine release profile and enhance microglial activation.* Neurosci Lett, 2014. **578**: p. 133-8.
32. Covas, D.T., et al., *Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts.* Exp Hematol, 2008. **36**(5): p. 642-54.
33. Katare, R., et al., *Transplantation of human pericyte progenitor cells improves the repair of infarcted heart through activation of an angiogenic program involving micro-RNA-132.* Circ Res, 2011. **109**(8): p. 894-906.
34. Crisan, M., et al., *Perivascular cells for regenerative medicine.* J Cell Mol Med, 2012. **16**(12): p. 2851-60.
35. Gubernator, M., et al., *Epigenetic profile of human adventitial progenitor cells correlates with therapeutic outcomes in a mouse model of limb ischemia.* Arterioscler Thromb Vasc Biol, 2015. **35**(3): p. 675-88.
36. Iacobazzi, D., et al., *Increased antioxidant defense mechanism in human adventitia-derived progenitor cells is associated with therapeutic benefit in ischemia.* Antioxid Redox Signal, 2014. **21**(11): p. 1591-604.
37. Avolio, E., et al., *Combined intramyocardial delivery of human pericytes and cardiac stem cells additively improves the healing of mouse infarcted hearts through stimulation of vascular and muscular repair.* Circ Res, 2015. **116**(10): p. e81-94.
38. Juchem, G., et al., *Pericytes in the macrovascular intima: possible physiological and pathogenetic impact.* Am J Physiol Heart Circ Physiol, 2010. **298**(3): p. H754-70.
39. Armulik, A., A. Abramsson, and C. Betsholtz, *Endothelial/pericyte interactions.* Circ Res, 2005. **97**(6): p. 512-23.
40. Dar, A., et al., *Multipotent vasculogenic pericytes from human pluripotent stem cells promote recovery of murine ischemic limb.* Circulation, 2012. **125**(1): p. 87-99.

41. Blocki, A., et al., *Not all MSCs can act as pericytes: functional in vitro assays to distinguish pericytes from other mesenchymal stem cells in angiogenesis.* Stem Cells Dev, 2013. **22**(17): p. 2347-55.
42. Nees, S., et al., *Isolation, bulk cultivation, and characterization of coronary microvascular pericytes: the second most frequent myocardial cell type in vitro.* Am J Physiol Heart Circ Physiol, 2012. **302**(1): p. H69-84.
43. Volz, K.S., et al., *Pericytes are progenitors for coronary artery smooth muscle.* Elife, 2015. **4**.
44. Kovacic, J.C., et al., *Epithelial-to-mesenchymal and endothelial-to-mesenchymal transition: from cardiovascular development to disease.* Circulation, 2012. **125**(14): p. 1795-808.
45. Cai, C.L., et al., *A myocardial lineage derives from Tbx18 epicardial cells.* Nature, 2008. **454**(7200): p. 104-8.
46. Mellgren, A.M., et al., *Platelet-derived growth factor receptor beta signaling is required for efficient epicardial cell migration and development of two distinct coronary vascular smooth muscle cell populations.* Circ Res, 2008. **103**(12): p. 1393-401.
47. Smith, C.L., et al., *Epicardial-derived cell epithelial-to-mesenchymal transition and fate specification require PDGF receptor signaling.* Circ Res, 2011. **108**(12): p. e15-26.
48. von Gise, A., et al., *WT1 regulates epicardial epithelial to mesenchymal transition through beta-catenin and retinoic acid signaling pathways.* Dev Biol, 2011. **356**(2): p. 421-31.
49. Wu, M., et al., *Epicardial spindle orientation controls cell entry into the myocardium.* Dev Cell, 2010. **19**(1): p. 114-25.
50. Fuxe, J., et al., *Pericyte requirement for anti-leak action of angiopoietin-1 and vascular remodeling in sustained inflammation.* Am J Pathol, 2011. **178**(6): p. 2897-909.
51. Goumans, M.J., et al., *Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors.* EMBO J, 2002. **21**(7): p. 1743-53.
52. Stratman, A.N., et al., *Endothelial-derived PDGF-BB and HB-EGF coordinately regulate pericyte recruitment during vasculogenic tube assembly and stabilization.* Blood, 2010. **116**(22): p. 4720-30.
53. Darland, D.C., et al., *Pericyte production of cell-associated VEGF is differentiation-dependent and is associated with endothelial survival.* Dev Biol, 2003. **264**(1): p. 275-88.
54. Chang, W.G., et al., *Pericytes modulate endothelial sprouting.* Cardiovasc Res, 2013. **100**(3): p. 492-500.
55. Tillet, E., et al., *N-cadherin deficiency impairs pericyte recruitment, and not endothelial differentiation or sprouting, in embryonic stem cell-derived angiogenesis.* Exp Cell Res, 2005. **310**(2): p. 392-400.
56. Tattersall, I.W., et al., *In vitro modeling of endothelial interaction with macrophages and pericytes demonstrates Notch signaling function in the vascular microenvironment.* Angiogenesis, 2016.
57. Ozerdem, U., et al., *NG2 proteoglycan is expressed exclusively by mural cells during vascular morphogenesis.* Dev Dyn, 2001. **222**(2): p. 218-27.
58. Bodnar, R.J., et al., *Pericyte regulation of vascular remodeling through the CXC receptor 3.* Arterioscler Thromb Vasc Biol, 2013. **33**(12): p. 2818-29.
59. Anastasia, A., et al., *Trkb signaling in pericytes is required for cardiac microvessel stabilization.* PLoS One, 2014. **9**(1): p. e87406.

60. Nees, S., et al., *Wall structures of myocardial precapillary arterioles and postcapillary venules reexamined and reconstructed in vitro for studies on barrier functions.* Am J Physiol Heart Circ Physiol, 2012. **302**(1): p. H51-68.
61. Juchem, G., et al., *Regulation of coronary venular barrier function by blood borne inflammatory mediators and pharmacological tools: insights from novel microvascular wall models.* Am J Physiol Heart Circ Physiol, 2012. **302**(3): p. H567-81.
62. Osterud, B. and E. Bjorklid, *Sources of tissue factor.* Semin Thromb Hemost, 2006. **32**(1): p. 11-23.
63. Jessup, M. and S. Brozena, *Heart failure.* N Engl J Med, 2003. **348**(20): p. 2007-18.
64. Corselli, M., et al., *Perivascular ancestors of adult multipotent stem cells.* Arterioscler Thromb Vasc Biol, 2010. **30**(6): p. 1104-9.
65. Chen, C.W., et al., *Human pericytes for ischemic heart repair.* Stem Cells, 2013. **31**(2): p. 305-16.
66. Yannarelli, G., et al., *Human umbilical cord perivascular cells exhibit enhanced cardiomyocyte reprogramming and cardiac function after experimental acute myocardial infarction.* Cell Transplant, 2013. **22**(9): p. 1651-66.
67. Spencer, H.L., et al., *A journey from basic stem cell discovery to clinical application: the case of adventitial progenitor cells.* Regen Med, 2015. **10**(1): p. 39-47.
68. Weiss, D.R., et al., *Extensive deendothelialization and thrombogenicity in routinely prepared vein grafts for coronary bypass operations: facts and remedy.* Int J Clin Exp Med, 2009. **2**(2): p. 95-113.
69. Liu, Y., et al., *Hepatocyte growth factor and c-Met expression in pericytes: implications for atherosclerotic plaque development.* J Pathol, 2007. **212**(1): p. 12-9.
70. Nakamura, Y., et al., *Expression of local hepatocyte growth factor system in vascular tissues.* Biochem Biophys Res Commun, 1995. **215**(2): p. 483-8.
71. Ivanov, D., et al., *Expression of cell adhesion molecule T-cadherin in the human vasculature.* Histochem Cell Biol, 2001. **115**(3): p. 231-42.
72. Keramati, A.R., et al., *Wild-type LRP6 inhibits, whereas atherosclerosis-linked LRP6R611C increases PDGF-dependent vascular smooth muscle cell proliferation.* Proc Natl Acad Sci U S A, 2011. **108**(5): p. 1914-8.
73. Siao, C.J., et al., *ProNGF, a cytokine induced after myocardial infarction in humans, targets pericytes to promote microvascular damage and activation.* J Exp Med, 2012. **209**(12): p. 2291-305.
74. O'Farrell, F.M. and D. Attwell, *A role for pericytes in coronary no-reflow.* Nat Rev Cardiol, 2014. **11**(7): p. 427-32.
75. Kirton, J.P., et al., *Wnt/beta-catenin signaling stimulates chondrogenic and inhibits adipogenic differentiation of pericytes: potential relevance to vascular disease?* Circ Res, 2007. **101**(6): p. 581-9.
76. Bobik, A., et al., *Distinct patterns of transforming growth factor-beta isoform and receptor expression in human atherosclerotic lesions. Colocalization implicates TGF-beta in fibrofatty lesion development.* Circulation, 1999. **99**(22): p. 2883-91.
77. Alexander, M.Y., et al., *Identification and characterization of vascular calcification-associated factor, a novel gene upregulated during vascular calcification in vitro and in vivo.* Arterioscler Thromb Vasc Biol, 2005. **25**(9): p. 1851-7.
78. Wilkinson, F.L., et al., *Contribution of VCAF-positive cells to neovascularization and calcification in atherosclerotic plaque development.* J Pathol, 2007. **211**(3): p. 362-9.
79. Weber, K.T., et al., *Myofibroblast-mediated mechanisms of pathological remodelling of the heart.* Nat Rev Cardiol, 2013. **10**(1): p. 15-26.

80. Kramann, R., et al., *Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis.* Cell Stem Cell, 2015. **16**(1): p. 51-66.
81. McCullough, P.A., A. Olobatoke, and T.E. Vanhecke, *Galectin-3: a novel blood test for the evaluation and management of patients with heart failure.* Rev Cardiovasc Med, 2011. **12**(4): p. 200-10.
82. Ieronimakis, N., et al., *Coronary adventitial cells are linked to perivascular cardiac fibrosis via TGFbeta1 signaling in the mdx mouse model of Duchenne muscular dystrophy.* J Mol Cell Cardiol, 2013. **63**: p. 122-34.
83. Wu, J., et al., *Origin of Matrix-Producing Cells That Contribute to Aortic Fibrosis in Hypertension.* Hypertension, 2016. **67**(2): p. 461-8.
84. Birbrair, A., et al., *Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner.* Stem Cell Res Ther, 2014. **5**(6): p. 122.